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**Short running title:** CO<sub>2</sub> raises whitefly's fertility

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**Feeding behavior, life history and virus transmission ability of *Bemisia tabaci* (Gennadius)  
Mediterranean species (Hemiptera: Aleyrodidae) under elevated CO<sub>2</sub>**

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**Abstract**

The continuous rise of CO<sub>2</sub> concentrations in the atmosphere is reducing plant nutritional quality for herbivores and indirectly affects their performance. The whitefly (*Bemisia tabaci*, Gennadius) is a major worldwide pest of agricultural crops causing significant yield losses. This study investigated the plant-mediated indirect effects of elevated CO<sub>2</sub> on the feeding behavior and life history of *B. tabaci* Mediterranean species. Eggplants were grown under elevated and ambient CO<sub>2</sub> concentrations for three weeks after which plants were either used to monitor the feeding behavior of whiteflies using the Electrical Penetration Graph technique or to examine fecundity and fertility of whiteflies. Plant leaf carbon, nitrogen, phenols and protein contents were also analyzed for each treatment. *Bemisia tabaci* feeding on plants exposed to elevated CO<sub>2</sub> showed a longer phloem ingestion and greater fertility compared to those exposed to ambient CO<sub>2</sub> suggesting that *B. tabaci* is capable of compensating for the plant nutritional deficit. Additionally, this study looked at the transmission of the virus *Tomato yellow leaf curl virus* (*Begomovirus*) by *B. tabaci* exposing source and receptor tomato plants to ambient or elevated CO<sub>2</sub> levels before or after virus transmission tests. Results indicate that *B. tabaci* transmitted the virus at the same rate independent to the CO<sub>2</sub> levels and plant treatment. Therefore, we conclude that *B. tabaci* Mediterranean species prevails over the difficulties that changes in CO<sub>2</sub> concentrations may cause and it is predicted that under future climate change conditions, *B. tabaci* would continue to be considered a serious threat for agriculture worldwide.

**Key words:** Carbon dioxide, eggplant, EPG, fitness, tomato, whiteflies

## Introduction

Since the industrialization period, the concentrations of greenhouse gases in the atmosphere have been significantly altered by the intensive use of fossil fuels and by deforestation. In particular, the emissions of carbon dioxide (CO<sub>2</sub>) have rapidly increased in the atmosphere varying from 280 ppm (parts per million) at the pre-industrialization period to 400 ppm indicated by recent data from the National Oceanic and Atmospheric Administration (NOAA, 2015). Moreover, the concentration of this gas is expected to increase considerably in the current century (IPCC, 2014) changing the physiology of plants and indirectly the behavior and performance of herbivores (Trębicki *et al.*, 2017a). Elevated CO<sub>2</sub> is known to accelerate plant growth and to increase plant photosynthetic rates, plant canopy temperatures, biomass and carbon:nitrogen (C:N) ratios (Ward & Kelly, 2004; Oehme *et al.*, 2011; Sun *et al.*, 2013; Curnutte *et al.*, 2014; Guo *et al.*, 2014; Kimball, 2016; Trębicki *et al.*, 2017a; Zhang *et al.*, 2018). Therefore, given that elevated CO<sub>2</sub> increases the carbohydrate accumulation and the generation of reactive oxygen species (ROS) in plant tissues and decreases nitrogen accumulation, soluble proteins and amino acids, the plant primary and secondary metabolites as well as antioxidant and enzymatic proteins are altered, and this is likely to impact the performance and behavior of herbivores (Guo *et al.*, 2014; Li *et al.*, 2017b; Rajashekar, 2018; Ryan *et al.*, 2014a; Sun *et al.*, 2013; Sun *et al.*, 2011; Trębicki *et al.*, 2017a).

Research has shown that the response of herbivores to elevated CO<sub>2</sub> varies depending upon the manner in which they feed. Some authors suggest that limited nitrogen concentration in plants treated with elevated CO<sub>2</sub> decreases fecundity, population abundance and growth rates of chewing insects and increases their development time and leaf consumption (Cornelissen, 2011; Guo *et al.*, 2014; Wang *et al.*, 2014; Zhang *et al.*, 2018). However, the effects of elevated CO<sub>2</sub> on sap sucking

insects are species-specific (Hughes & Bazzaz, 2001). For instance, the population abundance of *Aphis gossypii* (Glover), *Myzus persicae* (Sulzer) (Hughes & Bazzaz, 2001), *Rhopalosiphum maidis* (Fitch) (Xie *et al.*, 2014) or *Bemisia tabaci* (Gennadius) (Mediterranean species (MED) - Q biotype) (Li *et al.*, 2011) have been positively affected by elevated CO<sub>2</sub>. Some other sap feeders are unaffected such as *Aphis nerii* (Boyer de Fonscolombe), *Aphis oenotherae* (Oestlund) or *Aulacorthum solani* (Kaltenbach) (Hughes & Bazzaz, 2001). However, the increase of CO<sub>2</sub> levels has negatively altered the body mass of *Brevicoryne brassicae* (Linnaeus) (Klaiber *et al.*, 2013) and population abundance of *Acyrtosiphon pisum* (Harris) (Hughes & Bazzaz, 2001). In the case of *B. tabaci*, which is a major phloem feeding pest worldwide, little information is known regarding the effects of CO<sub>2</sub> on its feeding behavior and fitness (Curnutte *et al.*, 2014; Li *et al.*, 2017b; Li *et al.*, 2011; Sun *et al.*, 2011; Wang *et al.*, 2014).

*Bemisia tabaci* is one of the most serious agricultural pests worldwide because it is highly polyphagous, a vector of begomoviruses and rapidly develops insecticide resistance (Navas-Castillo *et al.*, 2011; Fang *et al.*, 2013; Gilioli *et al.*, 2014; Götz & Winter, 2016). It is considered a species complex with a minimum of 36 morphologically indistinguishable species of which the Middle East-Asia Minor 1 species (MEAM1 - B biotype) and the MED species are the most commonly known (Bellows *et al.*, 1994; De Barro *et al.*, 2011; Dinsdale *et al.*, 2010; Firdaus *et al.*, 2013). Both species could exhibit variable molecular features and biological behavior with respect to host range, plant virus transmission efficiency, the ability to cause phytotoxicity and the degree of fecundity and/or insecticide resistance considering the complexity of this species assembly (Brown *et al.*, 1995). Research on the effects of elevated CO<sub>2</sub> on *B. tabaci* MED species is limited since studies have been commonly conducted on the MEAM1 species (Sun *et al.*, 2011; Curnutte *et al.*, 2014). Elevated CO<sub>2</sub>

increases developmental time and population abundance (Sun *et al.*, 2011), without discernible impact on life-span, sex ratio, fecundity and density of MEAM1 species (Sun *et al.*, 2011; Wang *et al.*, 2014). Due to the differences between both species of whiteflies it is necessary to carry out further studies into the effects of increased CO<sub>2</sub> levels on the performance of *B. tabaci* MED species.

Whiteflies are important vectors of plant viruses (Hasegawa *et al.*, 2018; Li *et al.*, 2017a; Navas-Castillo *et al.*, 2011) and *B. tabaci* is vector of more than 200 species of plant viruses that cause significant losses in crops located from the tropics to the warmer temperate regions (Canto *et al.*, 2009). The *Tomato yellow leaf curl virus* (*Begomovirus*, TYLCV) is one of the main viruses transmitted by *B. tabaci* in tomato plants (*Solanum lycopersicum*, Linnaeus). Little information is known on the effects that elevated CO<sub>2</sub> could have on virus transmission by sap sucking insects. However, some studies showed that elevated CO<sub>2</sub> may increase the resistance of tomato plants to TYLCV, indicated by the decrease of virus incidence and infection severity (Huang *et al.*, 2012). It may also reduce *Cucumber mosaic virus* (*Cucumovirus*, CMV) transmission by *M. persicae* to pepper (*Capsicum annuum*, Linnaeus) receptor plants previously exposed to elevated CO<sub>2</sub> (Dáder *et al.*, 2016). Other authors indicated that elevated CO<sub>2</sub> activates plant defenses in tobacco plants (*Nicotiana tabacum*, Linnaeus) increasing plant metabolites after *M. persicae* and CMV infestation (Fu *et al.*, 2010) or *Potato virus Y* (*Potyvirus*, PVY) inoculation (Matros *et al.*, 2006). Nevertheless, the effects of CO<sub>2</sub> are also species-specific for viruses. Trębicki *et al.*, (2016) have demonstrated that development and fecundity of *Rhopalosiphum padi* (Linnaeus) on *Barley yellow dwarf virus* (BYDV) infected wheat plants, and its virus spread ability were unaffected by the increase of CO<sub>2</sub> levels. Similarly, the results of Bosquee *et al.* (2018) showed that the ability of *Myzus persicae* to spread PVY was unaffected by the level of CO<sub>2</sub> at short time frame and at different spatial scales. However, the same authors observed more efficient viral transmission under elevated CO<sub>2</sub> conditions, and it

was suggested that the main reason was the altered plant defenses or changes in the feeding behavior of their vector.

Given that atmospheric CO<sub>2</sub> levels are predicted to continue rising (IPCC, 2014), research that seeks to understand direct impacts on plant physiology, as well as direct and indirect effects on herbivores, such as *B. tabaci*, could help to improve pest management programs in a future climate change scenario. Therefore, the present study examines the feeding behavior (by means of the EPG technique) and fitness of *B. tabaci* MED species on eggplants that were previously exposed to elevated (700 ppm) and ambient (440 ppm) CO<sub>2</sub> concentrations. It was hypothesized that *B. tabaci* under elevated CO<sub>2</sub>, which increases plant biomass and C:N ratio (Ward & Kelly, 2004; Oehme *et al.*, 2011; Sun *et al.*, 2013; Curnutte *et al.*, 2014; Guo *et al.*, 2014), would change its performance and feeding behavior as suggested by Sun *et al.* (2011). Additionally, we also studied the effects of elevated CO<sub>2</sub> on TYLCV transmission by *B. tabaci* MED species on tomato plants.

## Materials and methods

### *Plant material, herbivore species and virus source*

Eggplants (*Solanum melongena*, Linnaeus) cv. Black Beauty were used to analyze the effects of elevated CO<sub>2</sub> on the fitness of whiteflies as colonies were reared on this plant. In this way, the effect of adaptation of the insect to a new host plant that could caused different results was avoided. Additionally, virus susceptible tomato plants (*S. lycopersicum*) cv. Marmande were used to analyze the effects of elevated CO<sub>2</sub> on the transmission of TYLCV by *B. tabaci* MED species (eggplants are not susceptible to TYLCV). Experiments were conducted at the Institute of Agricultural Sciences of the Spanish National Research Council (ICA-CSIC, Madrid, Spain) in two growth chambers with

different CO<sub>2</sub> regimes: ambient (aCO<sub>2</sub>; 440 ppm) and elevated CO<sub>2</sub> (eCO<sub>2</sub>; 700 ppm). Growing conditions were 24:20°C (day:night); RH: 66%; photoperiod: 14:10 h (light:dark) with three Philips Green Power LED Production Modules Deep Red/Blue 150 on each shelf and 200 µmol/m<sup>2</sup>/s light intensity at canopy level. Eggplants were sown in Petri dishes, transplanted into pots at seven days old (BBCH 7) (Acosta-Quezada *et al.*, 2016) and tomato plants were transplanted into pots at 10 days old (BBCH 10) with a mixture of soil : vermiculite (2 : 1). After transplanting, eggplants were transferred to each of the two growth chambers set at 700 or 440 ppm for three weeks. Plants were watered three times a week using 20-20-20 (N-P-K) Nutrichem fertilizer (Miller Chemical & Fertilizer Corp., Pennsylvania, USA).

*Bemisia tabaci* MED species were kindly provided from a colony maintained in the laboratory of Dr. Enrique Moriones at IHSM-La Mayora, CSIC (Málaga, Spain) in 2007. The population was reared on eggplants in greenhouse conditions (temperature ranges of 24 : 20 °C ± 2 (day : night); a photoperiod of 16 : 8 h (light : dark) with high-pressure sodium lights (Osram Plantastar 400 W E40) and relative humidity of 70%-80%. Adult whiteflies were separated to create a synchronized colony prior to the bioassay.

One month before the experiments, tomato plants were inoculated with TYLCV to create virus source plants for virus transmission experiments. *Bemisia tabaci* adults were collected from the main colony and placed in clip-cages previously installed on symptomatic leaves of TYLCV-infected tomato plants (30 adults per clip-cage). After the virus acquisition access period (72 h), the clip-cages with whiteflies were placed on leaves of healthy tomato plants (4-true leaf stage – BBCH 14). Leaves with clip-cages were cut after the inoculation period (72 h) and tomato plants were distributed into growth chambers with either elevated (700 ppm) or ambient (440 ppm) CO<sub>2</sub> treatments. Plants were then examined every three days to ensure the absence of whiteflies on the plants.



### *Whitefly fitness bioassay*

Eggplants (BBCH 14), previously grown over one month under elevated (700 ppm) or ambient (440 ppm) CO<sub>2</sub> conditions, were infested with ten couples of adult whiteflies with a clip-cage (2 cm of diameter) on the abaxial side of the youngest leaf of the plant. After 24 h clip-cages were removed and the development of ten eggs per plant was monitored in a climatic chamber (24:20°C (day:night); RH: 66%; photoperiod: 14 : 10 h (light : dark)) under ambient CO<sub>2</sub> conditions, until adult emergence. The emerged adult whiteflies were sexed, coupled and each couple was placed inside of a clip-cage on another leaf of the same plant for each of the treatments to evaluate fecundity and fertility of whiteflies for 30 days ( $n=20$ ).

### *Feeding behavior of *B. tabaci* using the Electrical Penetration Graph (EPG) technique*

Eggplants exposed to either ambient or elevated CO<sub>2</sub> levels were used to evaluate the feeding behavior of *B. tabaci*. Whitefly adult females were monitored using the Electrical Penetration Graph technique (EPG) for eight hours. Before the assay, a gold wire (20 mm length × 12.5 µm in diameter) glued to a thin copper wire (20 mm length) was attached to the pronotum of each whitefly with a tiny drop of water based silver-conducting glue paint (Rodríguez-López *et al.*, 2011). Whiteflies were starved for approximately one hour before the EPG test and were placed on the abaxial side of the second youngest leaf of each eggplant. The assay was conducted using eight channels (four plants for each treatment) of the Giga-Ohm DC-EPG device (EPG Systems, Wageningen, The Netherlands) until there were at least 20 recordings per treatment. A single adult whitefly and a plant were used for each replicate. After EPG recording, plants were collected and

introduced into a -80°C refrigerator for further analyses of protein and secondary metabolite content in plants. The EPG data was analyzed using Windows software Stylet+ (EPG Systems, Wageningen, The Netherlands) and the variables related to the feeding behavior of the whiteflies were processed using the EPG-Excel data Worksheet v.5.0 (Sarria *et al.*, 2009). The EPG recording was conducted at  $27 \pm 0.53^{\circ}\text{C}$ .

Twenty-eight EPG sequential and non-sequential variables were selected from the EPG-Excel data worksheet and compared between treatments as described in Backus *et al.* (2007): PPW (proportion of individuals that produced a specific waveform type), NWEI (number of waveform events per insect, that is the sum of the number of events of a particular waveform divided by the total number of insects under each treatment), WDI (total waveform duration (s) per insect, that is the sum of durations of each event of a particular waveform divided by the total number of insects under each treatment) and WDE (waveform duration (s) per event, that is the sum of the duration of the events for a particular waveform divided by the total number of events of that particular waveform under each treatment).

#### *TYLCV transmission experiments*

Two experiments were conducted to examine the effects of elevated CO<sub>2</sub> on TYLCV transmission by *B. tabaci* on tomato plants. In the first experiment (1) receptor plants were exposed to ambient and elevated CO<sub>2</sub> treatments during post-inoculation (3 weeks) and in the second experiment (2), receptor plants were under the two CO<sub>2</sub> treatments during pre and post-inoculation (5 weeks). Virus source plants were grown either under elevated or ambient CO<sub>2</sub> conditions. After the experiments, ten randomly selected plants were collected for each of the two treatments to measure the height, fresh weight and dry weight (dried at 80°C for 48 h).

(1) A month after the inoculation of the TYLCV source plants, around 750 whiteflies were placed on the virus source plant grown under the two different CO<sub>2</sub> treatments to acquire the virus (72h). Then five whiteflies were transferred to 3-week old tomato plants (BBCH 14) that were grown under ambient CO<sub>2</sub> and covered with cages of methacrylate cylinders for 72 h. Plants with insects were then transferred to either elevated or ambient CO<sub>2</sub> growth chambers and after the inoculation period, plants were treated twice with a systemic insecticide (Confidor 20 SL at 200 ppm dose applied at dropping point) to eliminate whiteflies. Plants infected with TYLCV were evaluated three to four weeks after inoculation. Positive infection was considered when visually plants presented clear symptoms (leaves are curled, yellow and stunted) (Fang *et al.*, 2013). These symptoms were correlated by PCR technique in previous experiments (Moreno-Delafuente *et al.*, 2013).

(2) Tomato receptor plants at cotyledon stage (BBCH 10) were distributed in the growth chambers with either 700 or 440 ppm of CO<sub>2</sub> treatments. One month after the inoculation of the virus source plants, the acquisition and inoculation process was performed as described in the first experiment (see above). After the inoculation, plants were transferred to their respective treatments.

#### *Plant analyses*

**Secondary metabolites analyses** Frozen samples (-80°C) were lyophilized to remove frozen water without liquid phase. Samples were analyzed for secondary metabolites by extraction of freeze-dried samples (30 mg) in 1 mL 70% methanol with shaking for 20 minutes, the sample was then centrifuged for 10 min at 10 000 r/min and the supernatant transferred to a clean tube. The pellet was extracted twice more with 0.5 mL 70% methanol. Methanol was evaporated using a Jouan RC1022 vacuum centrifuge (Thermo Scientific, Massachusetts, USA) before extracts were partially

purified by solid-phase extraction using a Sep-Pak Vac 500 mg C18 column (Waters Ltd., Elstree, UK) as described by Hauck *et al.* (2014). Samples were subsequently dried under vacuum at 60 °C and the dried pellets were resuspended in 500 µL 100% methanol and analyzed via high pressure liquid-chromatography with online photodiode array detection (HPLC-PDA) with a system comprising a Waters 515 pump, a Waters 717plus autosampler, a Waters 996 photodiode array detector and a Waters C<sub>18</sub> Nova-Pak radial compression column (C<sub>18</sub> 4.0 µm, 8.0 × 100 mm cartridge) (Waters Ltd., Elstree, UK) with an injection volume of 30 µL and a flow rate of 2 mL/min. The mobile phase consisted of 5% acetic acid (solvent A) and 100% methanol (solvent B) with a linear gradient from 5% to 75%, B in A, over 35 min. Peak integration was performed using the Empower software. Liquid chromatography with PDA and electrospray ionization-ion trap tandem mass spectrometry (LC-PDA-ESI/MS<sup>n</sup>) was performed to identify the major compounds. A Thermo Finnigan LC-MS system (Finnigan Surveyor LC pump plus, PDA plus detector, Finnigan LTQ linear ion trap) (Thermo Scientific, Massachusetts, USA) and a Waters Nova-Pak C<sub>18</sub> 4.0 µm, 3.9 × 100 mm column was used with an injection volume of 10 µL and a flow rate of 1 mL/min. The mobile phase consisted of purified water-0.1% formic acid (solvent A) and MeOH-0.1% formic acid (solvent B) with a linear gradient from 5% to 65%, B in A, over 60 min. Phenolics were characterized by UV absorption spectra, MS fragmentation patterns in negative ion mode and comparison with standards.

**Protein analysis** Samples were analyzed for proteins by extraction of freeze-dried samples (30 mg) in 1.8 mL McIlvaine buffer containing 50 mmol/L ascorbic acid and 0.2 mL 20% lithium deodecyl sulphate. Proteins were precipitated with 10% trichloroacetic acid, 0.2% phosphotungstic acid and resuspended in 0.1mol/L NaOH. Proteins were analyzed by the Lowry method as described by (Dáder *et al.*, 2014).

**Total carbon and nitrogen measurements** Total carbon and nitrogen content of four plants per treatment, were measured to link the effects of CO<sub>2</sub> on the virus transmission and plant characteristics with changes of plant C:N ratio in tomato plants. Plants were separated in roots, stem, leaves and flowers. All plant parts were dried for 24 h at 60°C and crushed to prepare the samples for the analyses. The analyses were performed using an Organic Elemental Analyzer - NC Soil Analyzer (Flash 2000, Thermo scientific) at ICA-CSIC (Madrid, Spain) and CEBAS-CSIC (Murcia, Spain).

#### *Statistical analysis*

All data were analyzed with SPSS Version 24.0 (Statistical Package for the Social Sciences or Statistical Product) (Carver & Nash, 2006). Raw data were checked for normality and homogeneity of variance using the Shapiro-Wilk W-test before performing the parametric test. Data were transformed with either  $\ln(x+1)$  or  $\arcsin\sqrt{x}$  if needed to reduce heteroscedasticity. The data were analyzed by a Student *t*-test ( $P < 0.05$ ) except the virus transmission data and total carbon and nitrogen content that were analyzed by a one-way ANOVA test and the means were subsequently separated using the Least Significant Difference (LSD) test. When data did not follow the ANOVA assumptions, a non-parametric Mann-Whitney U-test ( $P < 0.05$ ) was performed.

#### **Results**

Elevated CO<sub>2</sub> levels on eggplants indirectly affected some life history parameters of *B. tabaci*. The mean number of days from egg to adult of whiteflies on eggplants that were exposed to either elevated or ambient CO<sub>2</sub> levels was similar under both treatments ( $a\text{CO}_2 = 26.5 \pm 0.5$ ;  $e\text{CO}_2 = 26.0 \pm 0.6$ ;  $t = 0.686$ ;  $P = 0.498$ ). Although the number of hatched eggs per couple of *B. tabaci* ( $a\text{CO}_2 = 28.8 \pm$

6.5;  $eCO_2 = 29.5 \pm 5.3$ ;  $U = 145.5$ ;  $P = 0.804$ ) and fecundity ( $aCO_2$ :  $60.1 \pm 9.2$ ;  $eCO_2$ :  $53.7 \pm 8.2$ ;  $t = 0.520$ ;  $P = 0.607$ ) were similar under both treatments, egg fertility (%) was significantly greater on plants that were exposed to elevated  $CO_2$  ( $aCO_2$ :  $43.6 \pm 5.9$ ;  $eCO_2$ :  $62.0 \pm 6.8$ ;  $t = -2.040$ ;  $P = 0.049$ ) (Table 1).

Comparison of the feeding behavior of *B. tabaci* on eggplants exposed to either elevated  $CO_2$  ( $eCO_2$ ) or ambient  $CO_2$  ( $aCO_2$ ) concentrations are shown in Table 2. Several EPG variables showed that the feeding behavior of whiteflies differed when plants were raised under  $eCO_2$  or  $aCO_2$ . The duration of non-probe (np) (WDE:  $274.9 \pm 22.6$  s for  $eCO_2$  and WDE:  $227.6 \pm 14.8$  s for  $aCO_2$ ;  $U = 1002035$ ;  $P = 0.004$ ) and probe waveforms ( $159.4 \pm 9.5$  s for  $eCO_2$  and  $150.3 \pm 10.0$  s for  $aCO_2$ ;  $U = 925762$ ;  $P = 0.001$ ) was significantly higher on plants exposed to  $eCO_2$  than to  $aCO_2$ . The number ( $U = 138.5$ ;  $P = 0.022$ ) and duration ( $U = 148.5$ ;  $P = 0.052$ ) of the sustained phloem ingestion phase (E2s) was significantly lower for whiteflies feeding on eggplants exposed to  $eCO_2$  (NWEI:  $0.2 \pm 0.1$ ; WDI:  $399.6 \pm 228.6$  s) compared to those exposed to  $aCO_2$  (NWEI:  $0.6 \pm 0.2$ ; WDI:  $934.3 \pm 320.7$  s). Nevertheless, the whiteflies that were able to reach the phloem phase, spent more time ingesting phloem sap on plants previously exposed to  $eCO_2$  than on those exposed to  $aCO_2$ . This fact is shown by the duration per event (WDE) of E2s that was significantly higher ( $t = -2.246$ ;  $df = 14$ ;  $P = 0.041$ ) on whiteflies feeding on plants exposed to  $eCO_2$  (WDE:  $2664.0 \pm 541.4$  s) than to those exposed to  $aCO_2$  (WDE:  $1509.2 \pm 217.3$  s). Moreover, the number of whiteflies reaching the phloem phase and able to sustain phloem ingestion ( $E2 > 10$  min) was lower under  $eCO_2$  (3/7) than under  $aCO_2$  (10/12) (Table 2). The analysis of EPG sequential variables (Table 2) shows that  $eCO_2$  delays the time needed by the insect to start phloem phase activities. This is reflected by the following variables: time from the 1st probe to the 1st E ( $eCO_2$ :  $24867.7 \pm 1320.5$  s vs.  $aCO_2$ :  $19272.2 \pm 2115.4$  s;  $t = -2.218$ ;  $df = 39$ ;  $P = 0.032$ ), from the 1st probe to the 1st E2s ( $eCO_2$ :  $26736.6 \pm 962.1$  s vs.  $aCO_2$ :  $20834.8 \pm 2152.2$  s;  $t = -2.461$ ;  $df = 39$ ;  $P = 0.018$ ) and the time from the beginning of that probe to the 1st E2 ( $eCO_2$ :  $1258.3$

$\pm 279.6$  s vs. aCO<sub>2</sub>:  $695.2 \pm 111.5$  s;  $t = -2.272$ ;  $df = 14$ ;  $P = 0.039$ ). All these sequential variables gave significantly higher values when whiteflies fed on plants previously exposed to eCO<sub>2</sub> than to those exposed to aCO<sub>2</sub>. Also the number of probes after the 1<sup>st</sup> E was significantly lower on eggplants exposed to eCO<sub>2</sub> than to aCO<sub>2</sub> (NWEI:  $3.3 \pm 1.2$  for eCO<sub>2</sub>;  $14.8 \pm 4.3$  for aCO<sub>2</sub>;  $U = 138$ ;  $P = 0.041$ ).

Results of the TYLCV transmission assays indicate that CO<sub>2</sub> concentrations does not affect virus transmission rate to tomato plants by *B. tabaci*, showing no significant differences between both CO<sub>2</sub> treatments ( $F = 1.003$ ;  $df = 3$ ;  $P = 0.425$ ) (Table 3).

Additionally, plants exposed to eCO<sub>2</sub> showed altered physiology compared to those that were under aCO<sub>2</sub>. Tomato plants increased significantly in size under eCO<sub>2</sub> (aCO<sub>2</sub>:  $38.06 \pm 0.64$  cm; eCO<sub>2</sub>:  $48.25 \pm 0.87$  cm;  $F = 88.947$ ;  $P < 0.001$ ) and, therefore, plant fresh weight (g) was significantly higher compared to plants grown at aCO<sub>2</sub> levels (aCO<sub>2</sub>:  $20.46 \pm 1.35$  g; eCO<sub>2</sub>:  $23.76 \pm 1.48$  g;  $U = 154.000$ ;  $P = 0.006$ ).

Plants grown at eCO<sub>2</sub> showed a significant increase in soluble phenolic compounds (Figure 1). The specific phenols analyzed in the leaves were chlorogenic acid, two chlorogenic acid isomers, feruloyl quinate, quercetin rutinoside and kaempferol rutinoside. Chlorogenic acid was the main phenol presenting higher concentrations compared to the other phenols. Chlorogenic acid (eCO<sub>2</sub>:  $14.3e+6 \pm 0.7e+6$  mg/gDM vs. aCO<sub>2</sub>:  $9.8e+6 \pm 1.1e+6$  mg/gDM;  $U = 577$ ;  $P < 0.001$ ), chlorogenic acid isomer a (eCO<sub>2</sub>:  $32.4e+4 \pm 1.6e+4$  mg/gDM vs. aCO<sub>2</sub>:  $18.7e+4 \pm 2.9e+4$  mg/gDM;  $U = 325$ ;  $P < 0.001$ ), chlorogenic acid isomer b (eCO<sub>2</sub>:  $66.1e+4 \pm 3.0e+4$  mg/gDM vs. aCO<sub>2</sub>:  $66.8e+4 \pm 6.9e+4$  mg/gDM;  $U = 1182$ ;  $P = 0.160$ ), feruloyl quinate (eCO<sub>2</sub>:  $16.1e+4 \pm 0.8e+4$  mg/gDM vs. aCO<sub>2</sub>:  $10.1e+4 \pm 0.9e+4$  mg/gDM;  $U = 473$ ;  $P < 0.001$ ), quercetin rutinoside (eCO<sub>2</sub>:  $33.8e+4 \pm 2.2e+4$  mg/gDM vs. aCO<sub>2</sub>:  $17.6e+4 \pm 1.5e+4$  mg/gDM;  $U = 559$ ;  $P < 0.001$ ) and kaempferol rutinoside (eCO<sub>2</sub>:  $34.2e+4 \pm 3.0e+4$

mg/gDM vs. aCO<sub>2</sub>:  $13.2\text{e}+4 \pm 1.3\text{e}+4$  mg/gDM;  $U = 258$ ;  $P < 0.001$ ) significantly increased under eCO<sub>2</sub>. Additionally, the dry matter content of proteins significantly decreased in eggplants exposed to eCO<sub>2</sub> (aCO<sub>2</sub>:  $173.28 \pm 6.55$  mg/g; eCO<sub>2</sub>:  $130.46 \pm 4.68$  mg/g;  $t = 5.379$ ;  $df = 50$ ;  $P < 0.001$ ).

Elevated CO<sub>2</sub> changed the nitrogen content of both tomatoes and eggplants. Our results indicated that nitrogen (N) content in leaves was significantly reduced after exposure to elevated CO<sub>2</sub> concentrations. However, no significant differences were found in carbon (C) content or C/N ratio (Table 4).

## Discussion

The present study indicates that plants grown under elevated CO<sub>2</sub> alter the feeding behavior and fertility of *B. tabaci* MED species. The whiteflies reached phloem less number of times and phloem sap ingestion was delayed on plants that were previously exposed to elevated CO<sub>2</sub> levels than on those that were grown under ambient CO<sub>2</sub>. However, whiteflies that were indirectly affected by plants grown under elevated CO<sub>2</sub> levels showed longer episodes of sustained phloem ingestion which may have finally increased their fertility. However, TYLCV transmission was unaffected by the CO<sub>2</sub> treatments.

Herbivores feeding on phloem sap are expected to successfully overcome physical and chemical plant defenses before reaching the phloem (Guo *et al.*, 2014). It is known that CO<sub>2</sub> enrichment increases plant growth rates, biomass, leaf area index (Stiling *et al.*, 2002; Yan *et al.*, 2018) and also, it has been correlated with sizes and densities of foliar cell types (Del Toro *et al.*, 2017) which could be considered as physical barriers for sap-sucking insects. These herbivores have to penetrate the leaf with their stylets creating channels to salivate and consequently, ingest phloem



sap (Will *et al.*, 2007). *Bemisia tabaci* took a similar length of time to the first probe with both CO<sub>2</sub> treatments suggesting that there are no superficial factors that could affect probing behavior of the whitefly. On the other hand, the results obtained in the present study (Table 2) show that whiteflies need longer time to start phloem activities in plants exposed to elevated CO<sub>2</sub>. This fact could indicate that the feeding behavior of whiteflies could be affected by some type of physical or chemical barriers. Plant chemical defenses are regulated by the salicylic acid (SA), the jasmonic acid (JA) and ethylene (ET) signaling-pathways. Elevated CO<sub>2</sub> is known to alter SA and JA signaling-pathways increasing plant susceptibility to aphids (Sun *et al.*, 2013). However, Guo *et al.* (2014) indicated that the nitrogen fixation of host-plants is likely to vary the effectiveness of the SA signalling-pathway which may also affect the time that the pea aphid takes to reach the phloem of *Medicago truncatula* (Gaertn). In addition, the effect of CO<sub>2</sub> on JA may vary with plant type, thus Lu *et al.* (2018) demonstrated that elevated CO<sub>2</sub> in infected tobacco plants increased JA but decreased in infected rice plants. Based on these results, the JA levels in eggplant are more likely to increase as it is a Solanaceous plant, the same as tobacco and in turn, may increase the defenses of the eggplants against the whiteflies. Secondary metabolites are also considered as part of plant defense mechanisms against biotic and abiotic stresses and variations in composition are phenotypic responses associated with resource availability and pathogen attack (Matros *et al.*, 2006). Additionally, these metabolites are known to alter feeding behavior of different herbivores (Yan *et al.*, 2018). The main phenolic found in the eggplant leaves was chlorogenic acid which is generally the predominant soluble phenolic compound in the leaves of this cultivar (Whitaker & Stommel, 2003). This phenolic compound has been demonstrated to affect the digestion and infection of herbivores and pathogens respectively. Felton *et al.* (1989) tested the effects of chlorogenic acid on the larval growth of *Spodoptera exigua* (Hübner) feeding on *S. lycopersicum* and showed that this phenolic significantly inhibited the growth of this noctuid herbivore. Results of the present study

showed that whiteflies feeding on plants exposed to elevated CO<sub>2</sub> arrived less often to phloem and dedicated more time to starting phloem ingestion. This may indicate that chemical defenses of eggplants were enhanced with CO<sub>2</sub> making the plant more resistant to *B. tabaci* as phenolics increased and nitrogen content decreased. Nevertheless, once whiteflies reached the phloem sieve elements (only the 15% of the individuals tested with the EPG technique), they were able to sustain phloem sap uptake for a longer period and satisfy their nutritional requirements for successful performance.

Sap sucking insects are limited by phloem quality (Bezemer & Jones, 1998; Sun *et al.*, 2009) and elevated CO<sub>2</sub> is assumed to decrease quality of phloem for herbivores (Ryan *et al.*, 2014b; Sun *et al.*, 2013; Sun *et al.*, 2011). Some herbivores such as chewing insects are known to increase leaf damage under elevated CO<sub>2</sub> as a result of their ability to compensate for the nutritional deficit of the plants (Hughes & Bazzaz, 2001; Trębicki *et al.*, 2016; Zhang *et al.*, 2018). However, in addition to chewing insects, certain phloem feeders are found to increase sap ingestion as a compensatory feeding mechanism (Sun *et al.*, 2009; Trębicki *et al.*, 2016). Trębicki *et al.* (2016) observed that the bird cherry-oat aphid (*R. padi*) spent longer time in phloem sap ingestion on plants subjected to elevated CO<sub>2</sub>. Also, Sun *et al.* (2009) indicated that the cotton aphid (*A. gossypii*) excreted a greater amount of honeydew as a result of greater ingestion of phloem sap on Bt cotton plants grown under elevated CO<sub>2</sub> compared to plants exposed to ambient CO<sub>2</sub>. This compensatory feeding implicates a greater amount of phloem sap ingested by sap sucking insects that could result in greater plant damage on plants grown under elevated CO<sub>2</sub> compared to those under ambient CO<sub>2</sub>. Hughes and Bazzaz (2001) examined the effects of elevated CO<sub>2</sub> on the abundance of five aphid species. These authors found that the majority of aphids were not negatively affected by increasing CO<sub>2</sub> levels. The reasons suggested by these authors were that the decline of nitrogen and amino acid content in plants under elevated CO<sub>2</sub> conditions may be neutralized by other plant characteristics or changes in

the sugar soluble nitrogen ratio and, that those aphids were capable of compensating for the low plant nutritional quality by altering their feeding behavior. Studies have shown that elevated CO<sub>2</sub> changes the amino acid (nitrogen based compound) content of the plants by increasing minor amino acids but decreasing or unaltering major amino acids such as glutamine, glutamate, aspartate or alanine (Geiger *et al.*, 1998; Ryan *et al.*, 2015). Minor amino acids are synthesized via biosynthetic pathway using carbon skeletons while the pathway to form major amino acids is during nitrate assimilation (Geiger *et al.*, 1998). Also, some minor amino acids are known to act as phagostimulant for herbivores (Chapman, 2003). Therefore, the decrease in nitrogen content may have reduced major amino acids and the increase of the C:N ratio may have increased the availability of carbohydrates and carbon skeletons that stimulate the biosynthesis of minor amino acids. In the present study, results of plant biochemical analysis (Table 4) indicated that plant leaves contained significantly less nitrogen, higher C:N ratio and less dry matter protein after exposure to elevated CO<sub>2</sub>. This fact, could have stimulated minor amino acids synthesis and therefore, have modified the feeding behavior of the whitefly showing a longer duration of the sustained phloem ingestion phase (E2) (Table 2). However, further research is needed to evaluate the effects of minor amino acids on the feeding behavior of herbivores. Alternatively, there may be other mechanisms to overcome the nutritional deficit of plants exposed to elevated CO<sub>2</sub>. For instance, Sun *et al.* (2009) suggested that certain endosymbionts of *A. gossypii* might alter total amino acid composition to avoid the decrease of phloem nutritional quality. Moreover, a study of Sun *et al.* (2015) on *Acyrtosiphon pisum* feeding on *Medicago truncatula* indicated that elevated CO<sub>2</sub> reduced the stomatal aperture of plants. This induced an increase of phloem and xylem sap ingestion by *A. pisum* as a result of the decrease in transpiration and the increase in water potential of *M. truncatula*. Therefore, the increase of turgor of this plant species exposed to elevated CO<sub>2</sub> may also explain the enhanced performance of sap feeders. In contrast to these results, in the present study was not observed significant differences in

the total duration of the xylem sap ingestion when whiteflies were exposed to eggplants grown under ambient or elevated CO<sub>2</sub> which may be contingent on the host plant characteristics and herbivore species.

The decline in plant nutritional quality caused by elevated CO<sub>2</sub> is known to alter performance of herbivores (Ryan *et al.*, 2014b; Sun *et al.*, 2013; Sun *et al.*, 2011). However, contrary to what is expected, a meta-analysis evaluating the effects of elevated CO<sub>2</sub> on insect life history, showed that generally sap sucking herbivores improved their performance with elevated CO<sub>2</sub> (Robinson *et al.*, 2012), rejecting the general assumption that a decline in plant nutritional quality will necessarily reduce herbivore performance. Indeed, some authors have reported a positive impact of elevated CO<sub>2</sub> on population abundance of sap sucking insects such as *A. gossypii*, *M. persicae* (Hughes & Bazzaz, 2001), *B. tabaci* (Li *et al.*, 2011) and *R. padi* (Ryan *et al.*, 2015). However, the response to the increase of CO<sub>2</sub> is often species-specific as some phloem feeders such as *B. brassicae* (Klaiber *et al.*, 2013), *A. pisum* (Hughes & Bazzaz, 2001) and *Sipha flava* (Forbes, S.A.) (Auad *et al.*, 2012) were affected negatively by elevated CO<sub>2</sub> levels and others such as *A. nerii*, *A. oenotherae* and *A. solani* (Hughes & Bazzaz, 2001) were unaffected. In the present study, the whitefly *B. tabaci* MED species increased its fertility when feeding on plants exposed to elevated CO<sub>2</sub>. This indicates that *B. tabaci* is not only compensating for the nitrogen deficit of the plant but also over-compensates it, resulting in an increased number of offspring. These findings are supported by Li *et al.* (2011) where the population abundance of *B. tabaci* MED increased with elevated CO<sub>2</sub> levels feeding on nontransgenic cotton plants. However, in spite of the fact that nitrogen limitation in the plants could be fundamental for herbivore life-history on plants exposed to elevated CO<sub>2</sub>, little attention has been made to understand how this deficit could increase performance of phloem feeders. White (1984)

proposed the plant stress hypothesis where it was predicted that stressed plants are better hosts for herbivores. Plants exposed to elevated CO<sub>2</sub> caused nitrogen deficiency in the plants (Guo *et al.*, 2014; Wang *et al.*, 2014) stressing the plants and also inducing a reduction in the plant water potential in their leaves (Radin & Boyer, 1982). Consequently, plant susceptibility to phloem feeders such as *Bemisia argentifolii* (Gennadius) (Skinner, 1996) and *B. tabaci* is increased (Flint *et al.*, 1996; Hilje *et al.*, 2001). The nitrogen deficit could have had a stronger effect on the plant water management compared to the stomatal aperture mentioned above, as nitrogen deficiency also decreases the root hydraulic conductivity (Radin & Boyer, 1982). Ultimately, *B. tabaci* may be able to take advantage of the plant susceptibility and plant hydraulic conditions to improve fertility as reported in the present study.

Little attention has been paid to the impact of elevated CO<sub>2</sub> on virus transmission. The majority of research conducted in this topic has concluded that elevated CO<sub>2</sub> decreases virus transmission rate (Felton & Duffey, 1990; Matros *et al.*, 2006; Fu *et al.*, 2010; Huang *et al.*, 2012; Dáder *et al.*, 2016) as a consequence of the increase in secondary metabolites (Fu *et al.*, 2010; Matros *et al.*, 2006) such as chlorogenic acid (Felton & Duffey, 1990). However, Bosquee *et al.* (2018) suggested that the altered plant defenses or aphid feeding behavior may have increased the ability of *M. persicae* to transmit phytoviruses under elevated CO<sub>2</sub> conditions. Furthermore, Xie *et al.* (2014) indicated that the increase of alate abundance, as a result of the increase in CO<sub>2</sub> levels, may improve *R. maidis* migration and virus spread and Trębicki *et al.* (2017b) showed that in wheat plots grown under elevated CO<sub>2</sub> increased natural incidence of different viruses compared to those grown under ambient CO<sub>2</sub>, but *R. padi* on infected plants with *Barley yellow dwarf virus* (BYDV) were unaffected suggesting that elevated CO<sub>2</sub> will not delay or reduce the spread of this virus (Trębicki *et al.*, 2016). Despite the significant increase of phenolic compounds, especially chlorogenic acid, our results have indicated that the increase in CO<sub>2</sub> has no effect on the transmission of the TYLCV by *B.*

*tabaci*, similar to the findings of Trębicki *et al.* (2016). This result was not related to the number of TYLCV-DNA copies in virus acquisition or transmission, as previous studies have shown that the ability of virus transmission by *B. tabaci* is not affected by the changes in the number of TYLCV-DNA copies present in the body of the insect after virus acquisition from infected plants (Guo *et al.*, 2016). *Bemisia tabaci* has demonstrated the ability to overcome the presumable stress that elevated CO<sub>2</sub> could cause by spending longer time ingesting phloem sap in eggplants; however, this was not translated to a greater virus transmission rate in tomato plants under elevated CO<sub>2</sub> when compared to plants exposed to ambient CO<sub>2</sub>.

Results of the present study suggest that the two main species (MED – Q biotype and MEAM1 – B biotype) of *B. tabaci* may perform differently under elevated CO<sub>2</sub>. Recent studies indicated that elevated CO<sub>2</sub> on cotton increased developmental time and decreased survival ratio of *B. tabaci* MEAM1 species (Wang *et al.*, 2014) while oviposition and reproduction were unaffected on collard plants (*Brassica oleracea* ssp. *acephala*) (Curnutte *et al.*, 2014). However, in the present study *B. tabaci* MED species increased fertility and presumably future offspring on eggplants, which are similar results to those of Li *et al.* (2011) who reported an increase in the population abundance of this species when feeding on cotton. Therefore, these results suggest that eCO<sub>2</sub> concentration would benefit population increase of the MED species but would be detrimental to the MEAM1 species of the *B. tabaci* complex. If such is the case, the MED species would displace the MEAM1 species in the near future because of the predicted increasing concentrations of CO<sub>2</sub> in the atmosphere. Further research on the effects of climate change on the performance of *B. tabaci* should be studied for both species separately, to ensure the proper understanding of their behavior and fitness under future climate conditions.

Based on the results presented here, elevated CO<sub>2</sub> changes plant biochemical composition, feeding behavior and fitness parameters of *B. tabaci* MED species. It was also demonstrated that this whitefly species is capable of overcoming the decline in plant nutritional quality caused by the elevated CO<sub>2</sub>, spending a longer period at each event, actively ingesting phloem of plants and consequently, increasing fertility. Therefore, *B. tabaci* may adapt better to alterations of abiotic factors compared to other herbivores as the climate continues changing. This may create new challenges in the management of *B. tabaci* populations under future climate change scenarios.

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#### Disclosure

The authors have declared that no competing interests exist.

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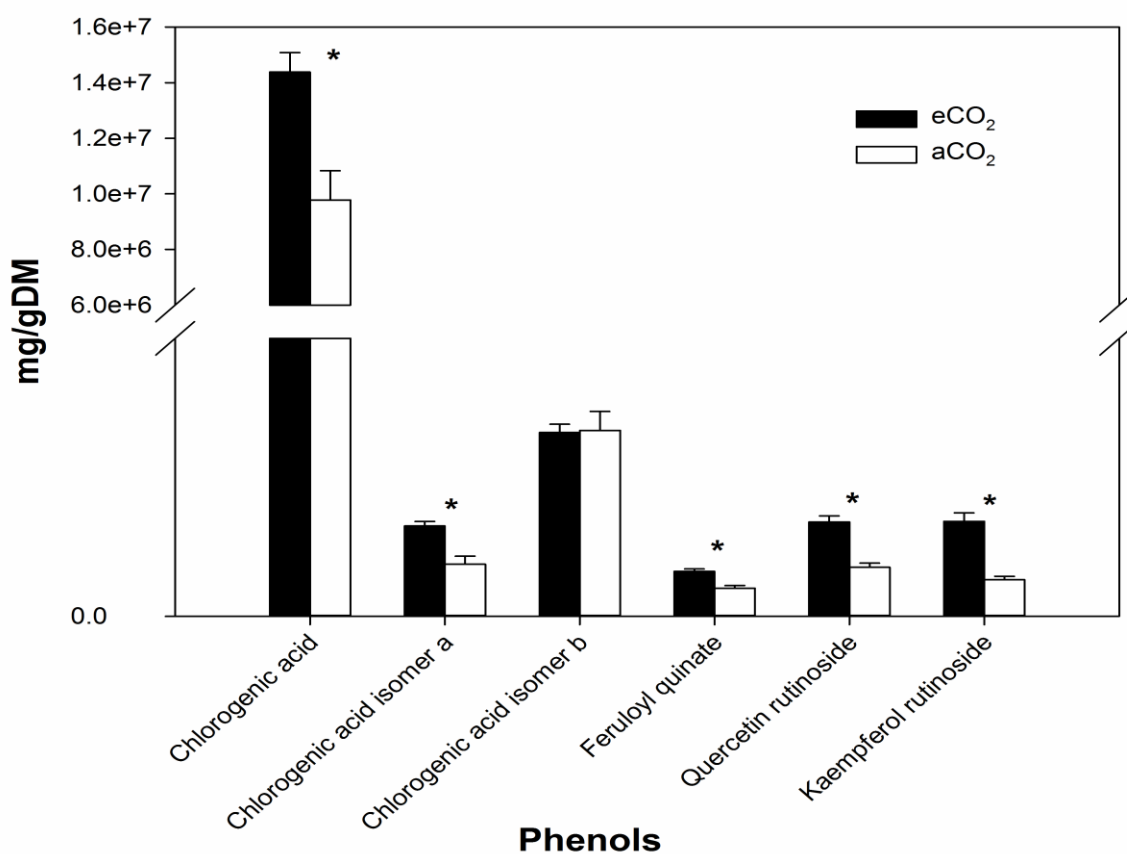
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## Figure legends

**Figure 1.** Mean  $\pm$  standard error values of the secondary metabolite analysis for ambient ( $a\text{CO}_2$ : 440 ppm) and elevated ( $e\text{CO}_2$ : 700 ppm)  $\text{CO}_2$  levels. The specific phenolic compounds observed in the leaves were chlorogenic acid, two chlorogenic isomers a & b, feruloyl quinate, quercetin rutinoside and kaempferol rutinoside. Statistical differences are calculated according Mann-Whitney U-test ( $P \leq 0.05$ ).



## Tables

**Table 1.** Mean  $\pm$  standard error values of the indirect effect of ambient (aCO<sub>2</sub>: 440 ppm) or elevated (eCO<sub>2</sub>: 700 ppm) CO<sub>2</sub> levels on different life parameters (days from eggs to adult, fertility, number of hatched eggs and fecundity) of *Bemisia tabaci* MED species on eggplants.

Life history parameters	aCO <sub>2</sub>	eCO <sub>2</sub>	Statistics	P-value
Days (from eggs to adult)	26.5 $\pm$ 0.5	26.0 $\pm$ 0.6	t = 0.686	0.498
Fecundity (number of eggs)	60.1 $\pm$ 9.2	53.7 $\pm$ 8.2	t = 0.520	0.607
Number of hatched eggs	28.8 $\pm$ 6.5	29.5 $\pm$ 5.3	U = 145.5	0.804
Fertility (% hatched eggs)	43.6 $\pm$ 5.9	62.0 $\pm$ 6.8	t = -2.040	0.049*

Statistical differences are calculated according to Student *t*-test for Gaussian variables or Mann-Whitney *U*-test for non-Gaussian variables ( $P \leq 0.05$ ).

**Table 2.** Mean  $\pm$  standard error values of non-sequential and sequential EPG variables for the probing behavior of *Bemisia tabaci* adult females on eggplants grown under 440 and 700 ppm of CO<sub>2</sub> concentrations. PPW: Proportion of individuals that produced the waveform type; NWEI: Number of waveform events per insect; WDI: Waveform duration (sec) per insect; WDE: Waveform duration (sec) per event.

Non-sequential variables	Treatment	PPW	NWEI	P	WDI	P	WDE	P
Non-probe	440	21/ 21	76.5 $\pm$ 8,0	0.352	17405.2 $\pm$ 728.7	0.441	227.6 $\pm$ 14.8	<u>0.004</u>
	700	20/ 20	66.5 $\pm$ 7,0		18280.2 $\pm$ 859.6		274.9 $\pm$ 22.6	
Probe	440	21 /21	75.8 $\pm$ 8.0	0.363	11394.8 $\pm$ 728.7	0.441	150.3 $\pm$ 10.0	<u>&lt;0.001</u>
	700	20/ 20	66.0 $\pm$ 7.0		10519.8 $\pm$ 859.6		159.4 $\pm$ 9.5	
Intercellular apoplastic stylet pathway (C)	440	21/ 21	77.2 $\pm$ 7.9	0.330	8954.5 $\pm$ 606.8	0.709	116.0 $\pm$ 4.4	<u>0.001</u>
	700	20/ 20	66.8 $\pm$ 6.9		9321.7 $\pm$ 770.4		139.6 $\pm$ 5.8	

Short intracellular punctures (pd)	440	17/ 21	4.2±0.7	0.134	24.2±4.4	0.965	4.7±0.3	0.627
	700	12/ 20	3.0±0.9		24.2±5.1		4.9±0.4	
Phloem phase (E)	440	12/ 21			1089.2±342.8	0.097		
	700	7/ 20			469.3±245.1			
Salivation into phloem sieve elements (E1)	440	12/ 21	1.0±0.3	0.121	51.3±17.7	0.328	51.3±11.8	0.186
	700	7/ 20	0.6±0.2		54.3±26.2		98.8±31.8	
Passive phloem sap uptake from the SE (E2)	440	12/ 21	1.0±0.3	<u>0.015</u>	1037.9±333.3	0.155	1037.9±190.0	0.794
	700	4/ 20	0.4±0.2		436.8±245.5		1185.5±561.4	
Sustained E2 (>10 minutes) (E2s)	440	10/ 21	0.6±0.2	<u>0.022</u>	934.3±320.7	0.052	1509.2±217.3	<u>0.041</u>
	700	3/ 20	0.2±0.1		399.6±228.6		2664.0±541.4	
Active intake of xylem sap (G)	440	10/ 21	0.7±0.2	0.286	1351.1±382.1	0.173	1891.6±334.7	0.358
	700	6/ 20	0.5±0.2		728.9±402.5		1457.7±307.1	
Probe to 1 <sup>st</sup> E1	440	12/ 21	37.3±7.8	0.772				
	700	7/ 20	40.6±5.1					
Probe after 1 <sup>st</sup> E	440	12/ 21	14.8±4.3	<u>0.041</u>				
	700	7/ 20	3.3±1.2					
np after the probe of the 1 <sup>st</sup> E2s	440	10/ 21			770.8±320.1	0.499		
	700	3/ 20			751.2±289.4			
<b>Sequential variables</b>								
Start of EPG to 1 <sup>st</sup> probe	440	21/ 21			324.8±80.1	0.602		
	700	20/ 20			459.3±135.0			
1 <sup>st</sup> probe to 1 <sup>st</sup> E	440	21/ 21			19272.2±2115.4	<u>0.032</u>		
	700	20/ 20			24867.7±1320.5			

Beginning of that probe to 1 <sup>st</sup> E	440	12/ 21	661.1±112.6	0.510
	700	7/ 20	818.5±240.7	
Beginning of that probe to 1 <sup>st</sup> E2	440	12/ 21	695.2±111.5	<u>0.039</u>
	700	4/ 20	1258.3±279.6	
1 <sup>st</sup> probe to 1 <sup>st</sup> E2s	440	21/ 21	20834.8±2152.2	<u>0.018</u>
	700	20/ 20	26736.6±962.1	
Beginning of that probe to 1 <sup>st</sup> E2s	440	10/ 21	851.7±144.0	0.565
	700	3/ 20	1020.2±176.1	

P-values are recorded according to Mann Whitney *U*-test for non-Gaussian distribution variables and to the *t*-test for parametric variables. Underline-type indicates significant differences ( $P \leq 0.05$ ).

**Table 3.** Effect of elevated CO<sub>2</sub> on TYLCV transmission rate (Mean ± SE) by *B. tabaci* on tomato plants

Receptor	Source	Mean±SE	P-value
aCO2	aCO2	66.5±5.6	0.425
eCO2	aCO2	55.5±8.5	
aCO2	eCO2	54.5±6.9	
eCO2	eCO2	51.3±5.5	

Differences were statistically compared by one way ANOVA ( $P \leq 0.05$ ). The transmission efficiency data were transformed as an arcsinVx prior to analysis. Each transmission experiment was evaluated four times.

**Table 4.** Chemical profile of tomato leaf on TYLCV infected and non-infected plants and eggplant leaves under aCO<sub>2</sub> (440 ppm) and eCO<sub>2</sub> (700 ppm) conditions

Plant type		N (%)		C (%)		C/N	
		Mean±SE	<i>P</i>	Mean±SE	<i>P</i>	Mean±SE	<i>P</i>
Tomato	aCO <sub>2</sub> -non-infected plant	4.8±0.3 a	<u>0.010</u>	40.4±1.1 a	0.850	8.8±0.8 a	0.906
	aCO <sub>2</sub> -TYLCV	4.7±0.3 a		40.0±1.1 a		8.9±1.7 a	
	eCO <sub>2</sub> -non-infected plant	3.3±0.4 b		39.5±0.8 a		13.2±1.7 a	
	eCO <sub>2</sub> -TYLCV infected plant	3.9±0.3 ab		39.3±0.5 a		10.4±0.7 a	
Eggplant	aCO <sub>2</sub>	7.0±0.1	<u>0.000</u>	36.9±0.2	0.545	5.3±0.1	<u>0.000</u>
	eCO <sub>2</sub>	5.7±0.2		36.8±0.3		6.6±0.3	

Statistical differences were analyzed according to ANOVA for Gaussian variables ( $P \leq 0.05$ ) and according to Mann Whitney *U*-test for non-Gaussian distribution variables. Different letters within columns indicate significant differences ( $P \leq 0.05$ ).